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Supporting Information

Observing Changes in the Structure and Oligomerization State of a Helical Protein Dimer using Solid-State Nanopores

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Peptide Synthesis:

Piperidine (Sigma-Aldritch, St. Louis MO) in N,N-dimethylformamide (DMF) (EMD Millipore, Billerica MA) was used as the deprotection agent solution, 0.5M [benzotriazol-1-yloxy(dimethylamino)-methylidene]-dimethyl-azanium hexafluorophosphate (HBTU) (EMD Chemicals) in DMF (EMD Chemicals) was used as the activator; 2M diisopropylethylamine (DIPEA) (Sigma-Aldritch) in N-methyl-2-pyrrolidone (NMP) (EMD Chemicals) was used as the activator base. 5-molar equivalents of the amino acid were used for each coupling.

For peptide GCN4-p1 NovaSyn TGR resin (0.25 mmol/g subsition, Novabiochem) was used. Methods 1 to 5 appear below. Arg33 was coupled using Method 5, Glu32, Val30, Leu29, Val23, Glu22, Ser14, Leu13, Val9, K8, D7, E6, L5, Q4, K3, M2 were coupled with Method 2, Arg1, Arg5 were coupled using Method 3, His18 was coupled using Method 4, all other residues were coupled with Method 1. The N-terminus of peptide GCN4-p1 was acetylated with 10% acetic anhydride (EMD), 6% N-methylmorpholine (NMM) (Sigma-Aldritch) in DMF (EMD Millipore).

The following FMOC amino-acids were used and purchased from Novabiochem; Fmoc-Lys(Boc)-OH, Fmoc-Gly-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Val-OH, Fmoc-Leu-OH, Fmoc-Ala-OH, Fmoc-Asn(Trt)-OH, Fmoc-His(Trt)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Gln(Trt)-OH, and Fmoc-Met-OH.

Method 1: Initial 30 sec. microwave deprotection (40W, 75º C), followed by 3 min microwave deprotection (40W, 75ºC). A single microwave coupling was performed for 5min (25W, 75º C).

Method 2: Initial 30 sec. microwave deprotection (40W, 75º C), followed by 3 min microwave deprotection (40W, 75ºC). Two consecutive microwave couplings were performed for 5 min (25W, 75º).

Method 3: Initial 30 sec. microwave deprotection (40W, 75º C), followed by 3 min microwave deprotection (40W, 75ºC). Two consecutive coupling cycles were used. The first coupling was at room temperature for 25 min followed by an additional 5 min under microwave power (25W, 75º C). The second coupling was performed under microwave power for 5min (25W, 75º C).

Method 4: Initial 30 sec. microwave deprotection (40W, 75º C), followed by 3 min microwave deprotonation (40W, 75ºC). A single microwave coupling was performed for 5min (25W, 50º C).

Method 5: Deprotection steps were not used to load the unprotected resin. Two consecutive coupling cycles were performed. The first coupling was at room temperature for 25 min followed by an additional 5 min under microwave power (25W, 75º C). The second coupling was performed under microwave power for 5min (25W, 75º C).

CD Spectroscopy

CD Spectroscopy was performed on peptide GCN4-p1 using an AVIV Model 410 Circular Dichroism Spectrophotometer (AVIV Biomedical, Lakewood NJ). The concentrations of the peptide stock solution 50 μM was determined using UV absorbance at 280 nm, (ε = 1330), and diluted to the appropriate concentration in buffer.

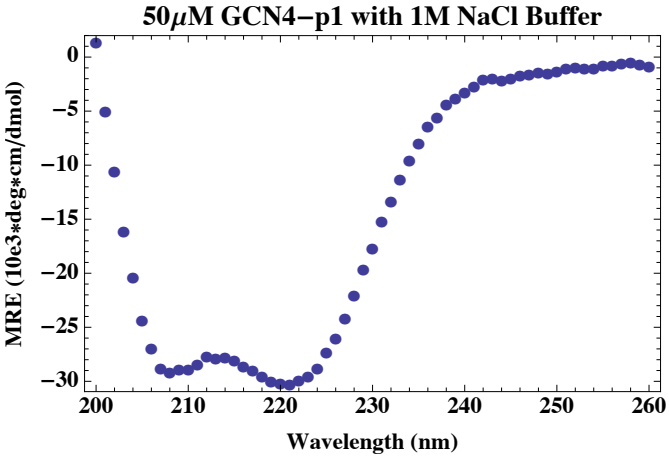

Figure S1: CD measurements on the GCN4-p1 peptide. Solution was 50 μM GCN4-p1 with 1M NaCl and phosphate buffer. Wavelength scans were run at 21º C, starting from 260 nm – 200 nm with a 1 nm step size and 15 sec. averaging time.

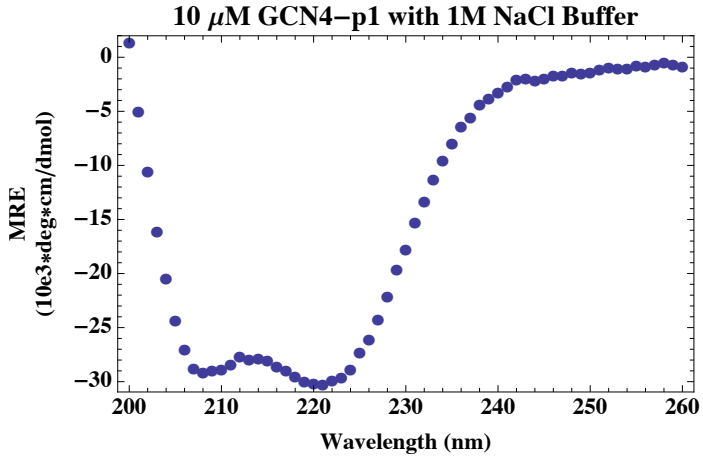

Figure S2: CD measurements on the GCN4-p1 peptide. Solution was 10 μM GCN4-p1 with 1M NaCl and phosphate buffer. Wavelength scans were run at 21° C, starting from 260 nm – 200 nm with a 1 nm step size and 15 sec. averaging time.

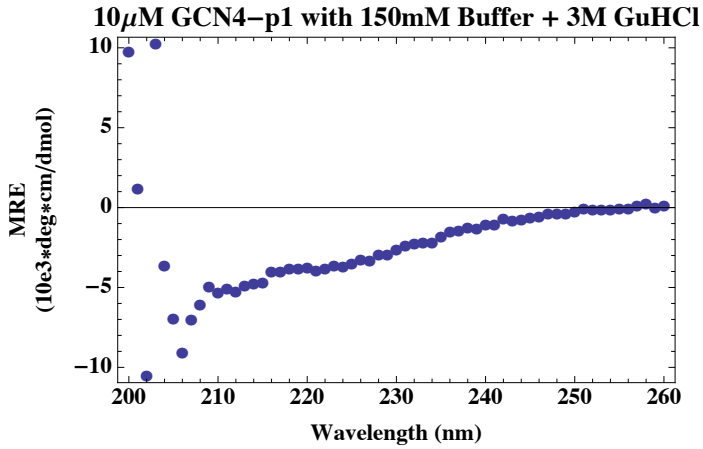

Figure S3: CD measurements on the GCN4-p1 peptide. Solution was 10 μM GCN4-p1 with 150 mM NaCl and phosphate buffer and 3M GdHCl. Wavelength scans were run at 25° C, starting from 260 nm – 200 nm with a 1 nm step size and 15 sec. averaging time.

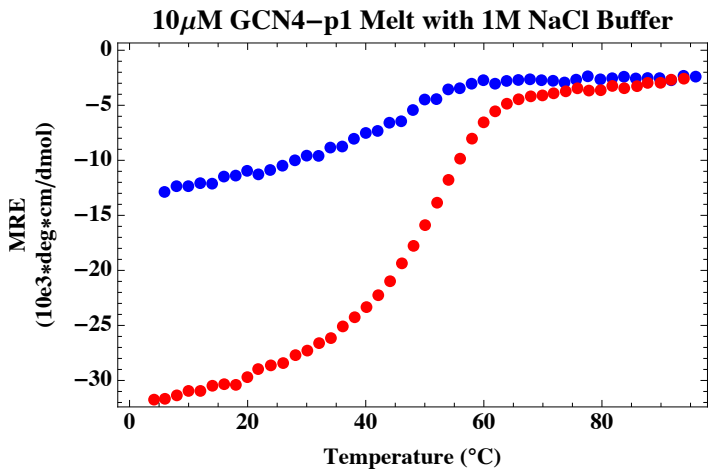

Figure S4: CD measurements on the GCN4-p1 peptide. Solution was 10 μM GCN4-p1 with 1M NaCl and phosphate buffer. Temperature dependence of Mean Residue Ellipticity (MRE) at 222 nm (with 15 second averaging time) is plotted against measured temperatures ranging from 4º C to 96º C, with a 1 min equilibration time between temperature steps and a 5 min wait time prior to the reverse scans. Red points indicate rising temperature. Blue points indicate lowering temperature.

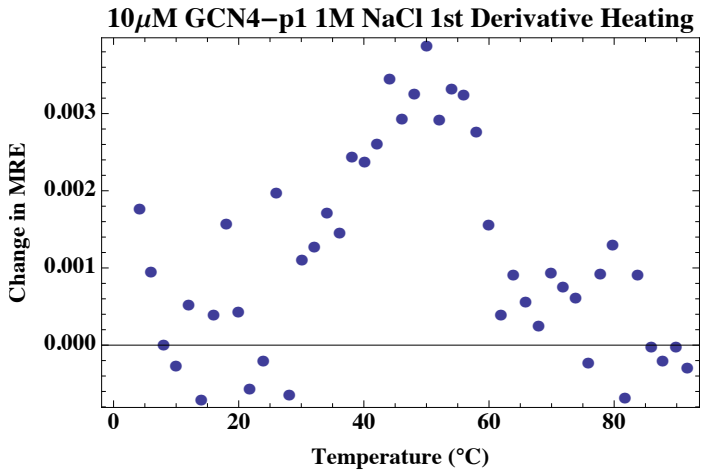

Figure S5: CD measurements on the GCN4-p1 peptide. Solution was 10 μM GCN4-p1 with 1M NaCl and phosphate buffer. The rate of change in Mean Residue Ellipticity (MRE) at 222 nm (with 15 second averaging time) is plotted against measured

temperatures ranging from 4º C to 96º C. Temperature of maximum rate of change provides an estimate of T_m = 54 °C. "Change in MRE" is calculated as the difference in MRE at 222 nm at adjacent temperatures divided by the difference in the two temperatures.

Control at positive voltage.

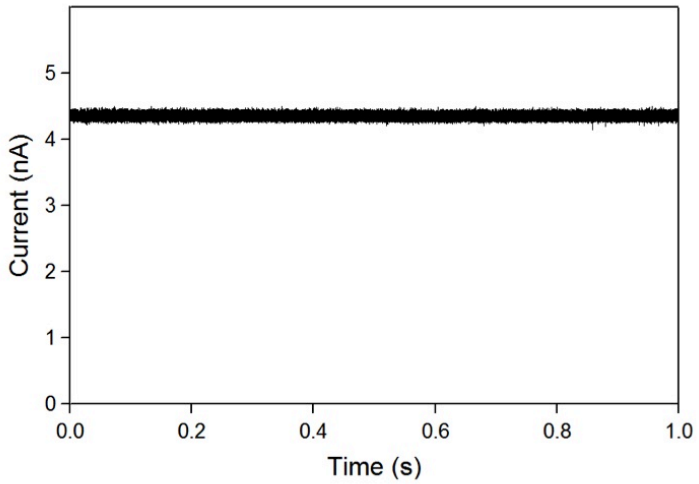

Figure S6: Control trace showing no GCN4-p1 induced events at positive voltage polarity. Solution was 1M NaCl, 6μM GCN4-p1, 50 mM potassium phosphate buffer. Applied voltage was +300 mV. Nanopore diameter was 6 nm.

Event signals at different GCN4-p1 concentrations.

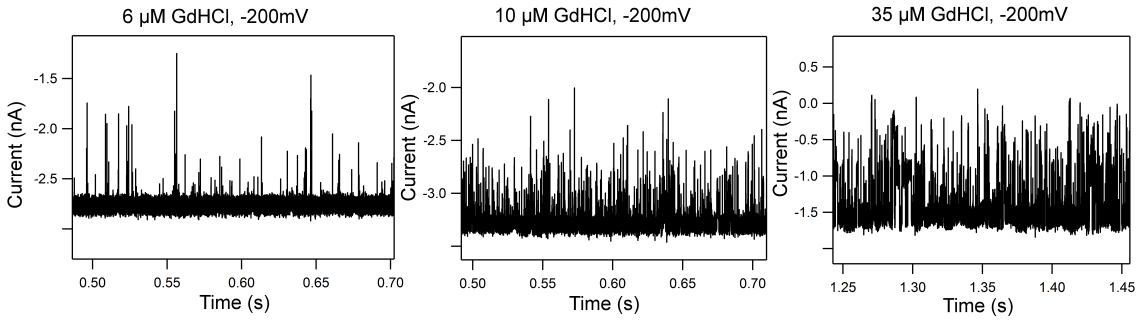

Figure S7: GCN4-p1 events at different concentrations. Representative traces of GCN4-p1 induced events at -200 mV are given for GCN4-p1 concentrations of 6 µM, 10 µM, and 35 µM. In each case GCN4-p1 was added to 1M NaCl, 50 mM potassium phosphate buffer, pH 7.0.

Dwell-time analysis of GCN4-p1 events with and without added GdHCl:

Dwell-time comparisons with and without 3M GdHCl added to solution are shown in **Figure S8**. For all voltages measured, dwell-times decreased substantially with the 3M GdHCl denaturing agent.

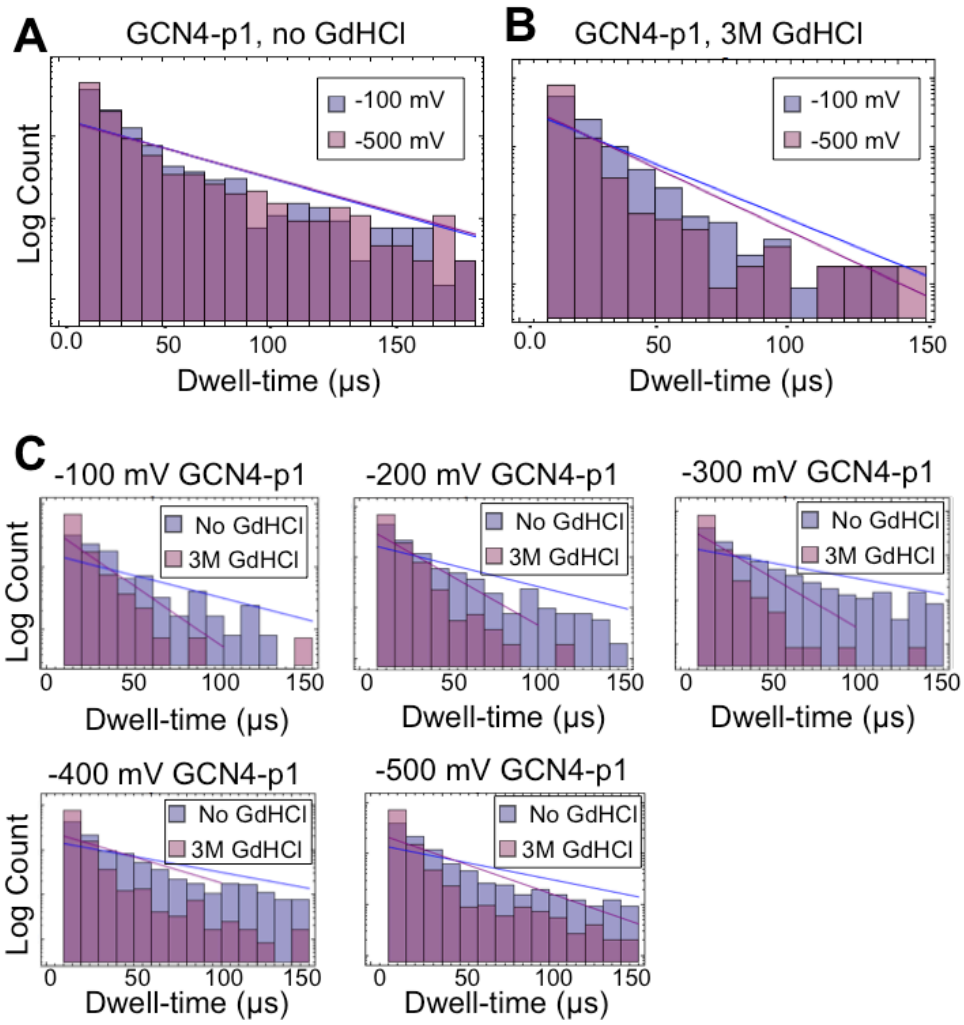

Figure S8: Comparison of measured GCN4-p1 event dwell-times. **(a)** Direct comparison of dwell-time histograms for GCN4-p1 events at 1M NaCl, 50 mM potassium phosphate, pH 7.0 for both -100 mV and -500 mV applied voltage. A slight reduction in dwell-time is observed for the higher voltage. **(b)** Direct comparison of dwell-time histograms for GCN4-p1 events at 3M GdHCl, 1M NaCl, 50 mM potassium phosphate, pH 7.0 for both -100 mV and -500 mV applied voltage. A reduction in dwell-time is observed for the

higher magnitude voltage. **(c)** Direct comparison of dwell-time histograms for GCN4-p1 events at 1M NaCl, 50 mM potassium phosphate, pH 7.0, both with and without 3M GdHCl added no solution. Voltages of -100 mV, -200mV, -300 mV, -400 mV, -500 mV are displayed. Dwell-times are consistently shorter for GCN4-p1 in 3M GdHCl solutions.

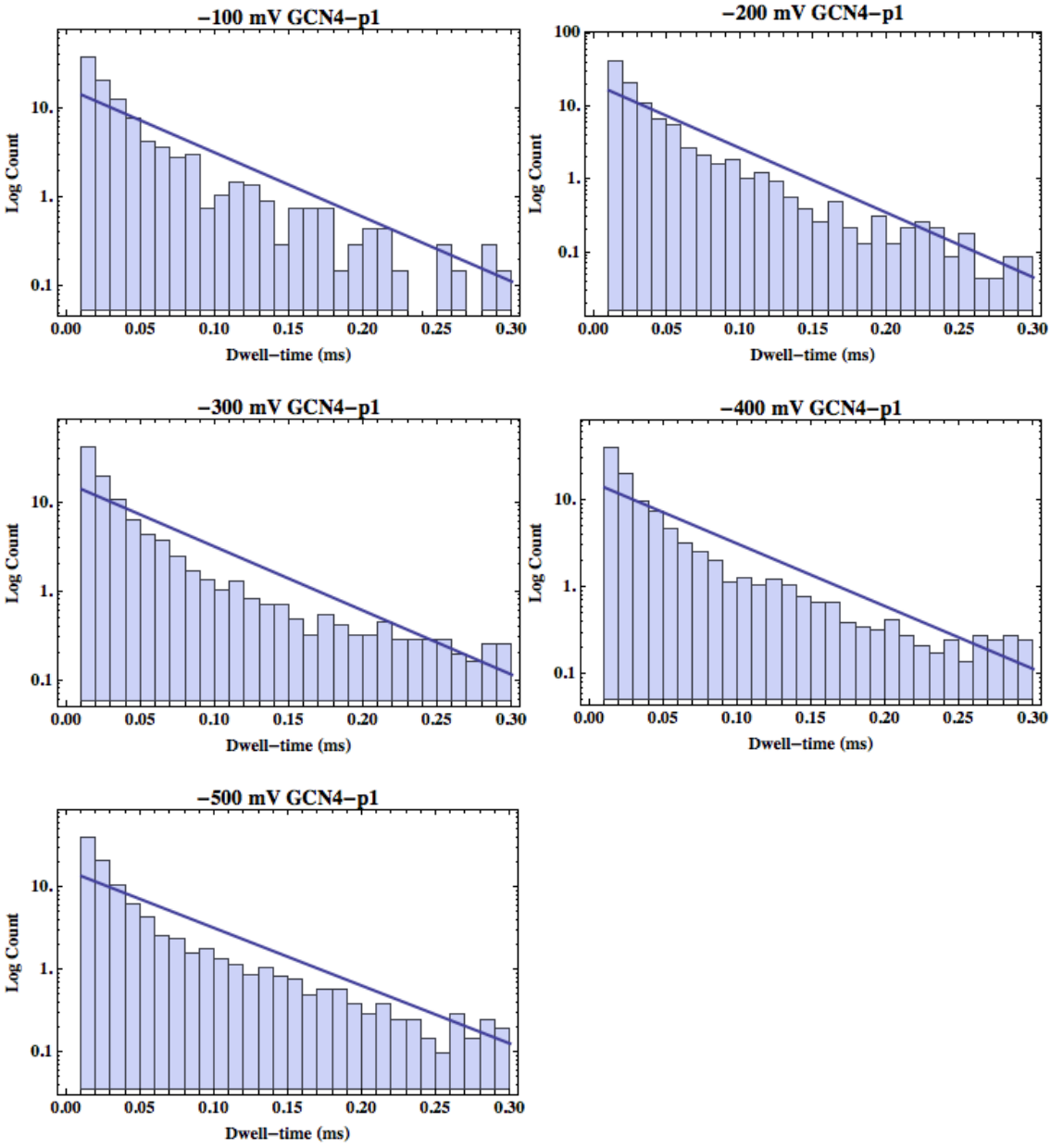

Figure S9: Representative histograms of dwell-time for GCN4-p1. Dwell-time histograms for GCN4-p1 events at 1M NaCl, 50 mM potassium phosphate, pH 7.0 for -100 mV, -200 mV, -300 mV, -400 mV, and -500 mV are shown. Forced fits to single-term exponential decay functions are plotted as solid lines.

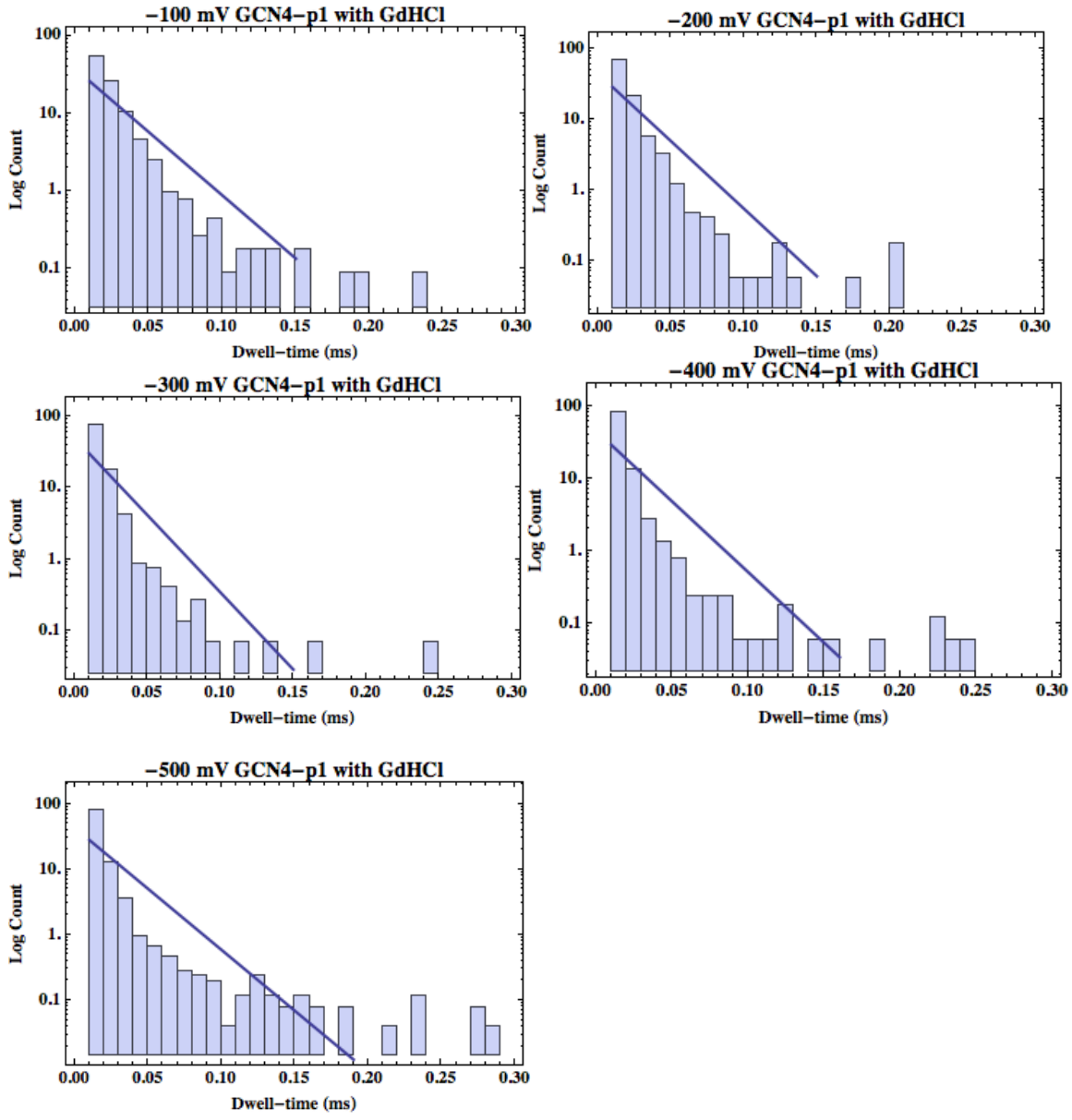

Figure S10: Representative histograms of dwell-time for GCN4-p1 with GdHCl. Dwell-time histograms for GCN4-p1 events at 1M NaCl, 3 M GdHCl, 50 mM potassium

phosphate, pH 7.0 for -100 mV, -200 mV, -300 mV, -400 mV, and -500 mV are shown. Forced fits to single-term exponential decay functions are plotted as solid lines.

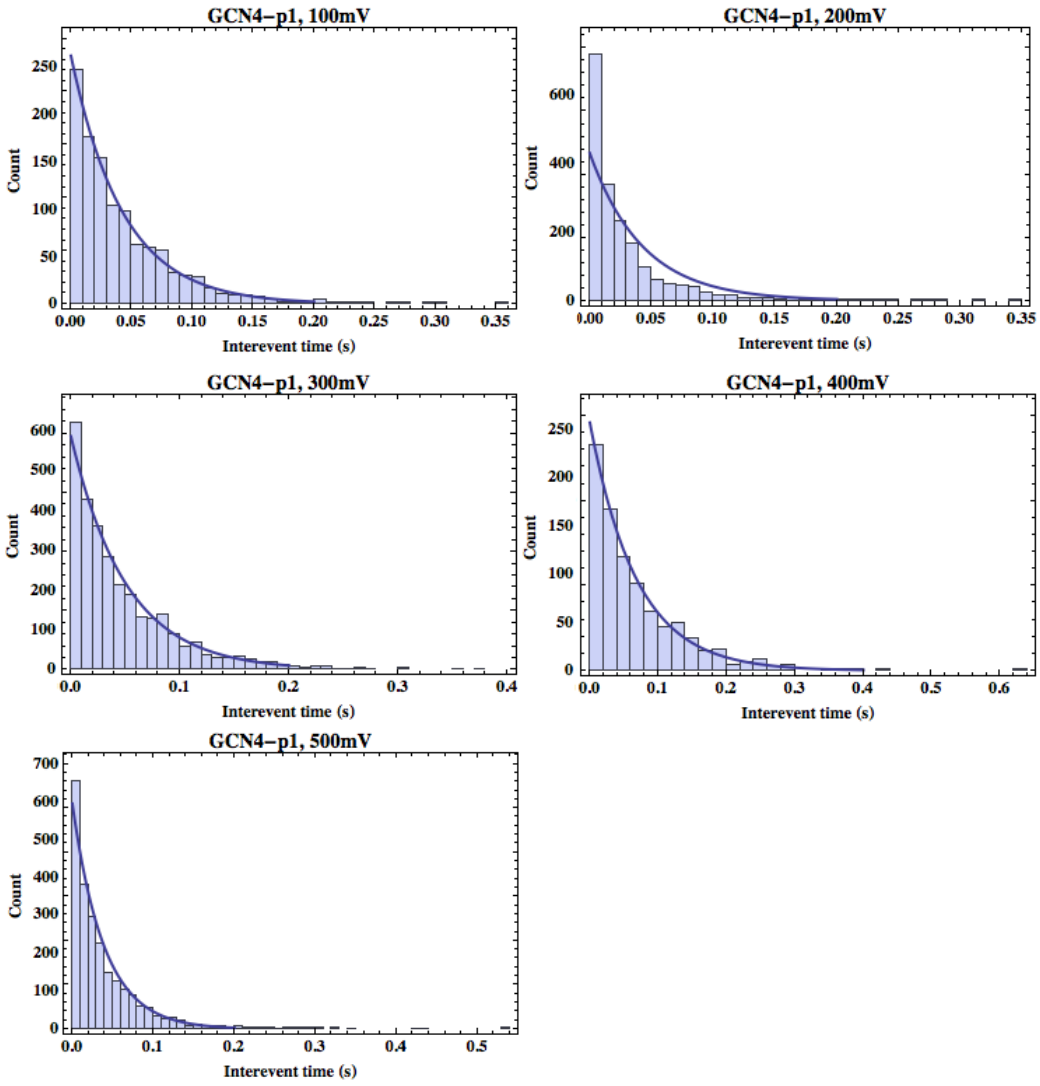

Figure S11: Representative histograms of inter-event time for GCN4-p1. The inter-event time histograms for GCN4-p1 events at 1M NaCl, 50 mM potassium phosphate, pH 7.0 are plotted for applied voltages of -100 mV, -200 mV, -300 mV, -400 mV, and -500 mV. All traces except that at -200 mV could be fit to a single-term exponential decay functions, which are plotted as solid lines.

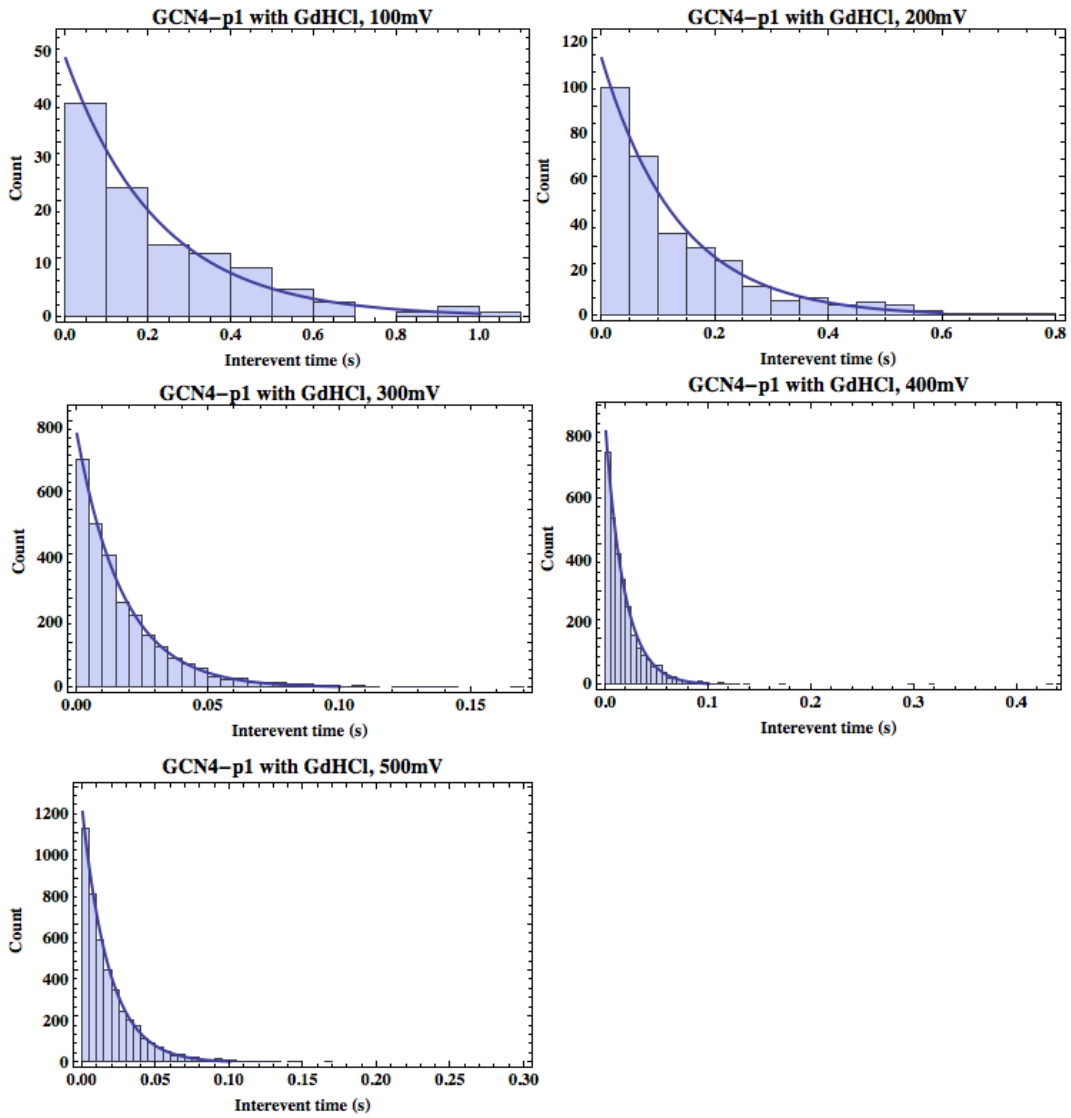

Figure S12: Representative histograms of inter-event time for GCN4-p1 with GdHCl. The inter-event time histograms for GCN4-p1 events at 1M NaCl, 3M GdHCl, 50 mM potassium phosphate, pH 7.0 are plotted for applied voltages of -100 mV, -200 mV, -300 mV, -400 mV, and -500 mV. All traces could be fit to single-term exponential decay functions, which are plotted as solid lines.

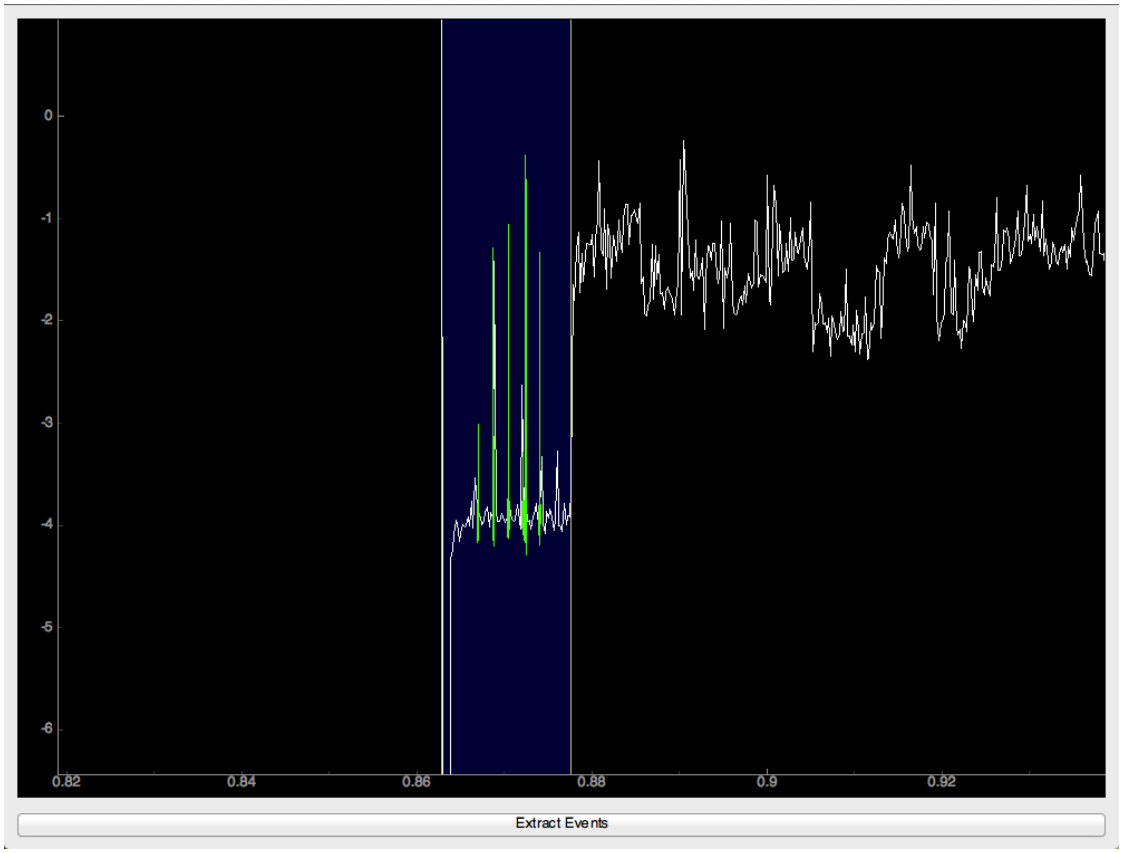

Figure S13: Selecting events from high temperature traces. The image is a screen capture from Pypore search and analysis software. The trace shows a "clogging event" from a baseline current of -4 nA to the clogged current ~ -1.5nA. The dark blue area represents the portion of the trace that was used in analysis. The green lines show events found by Pypore.

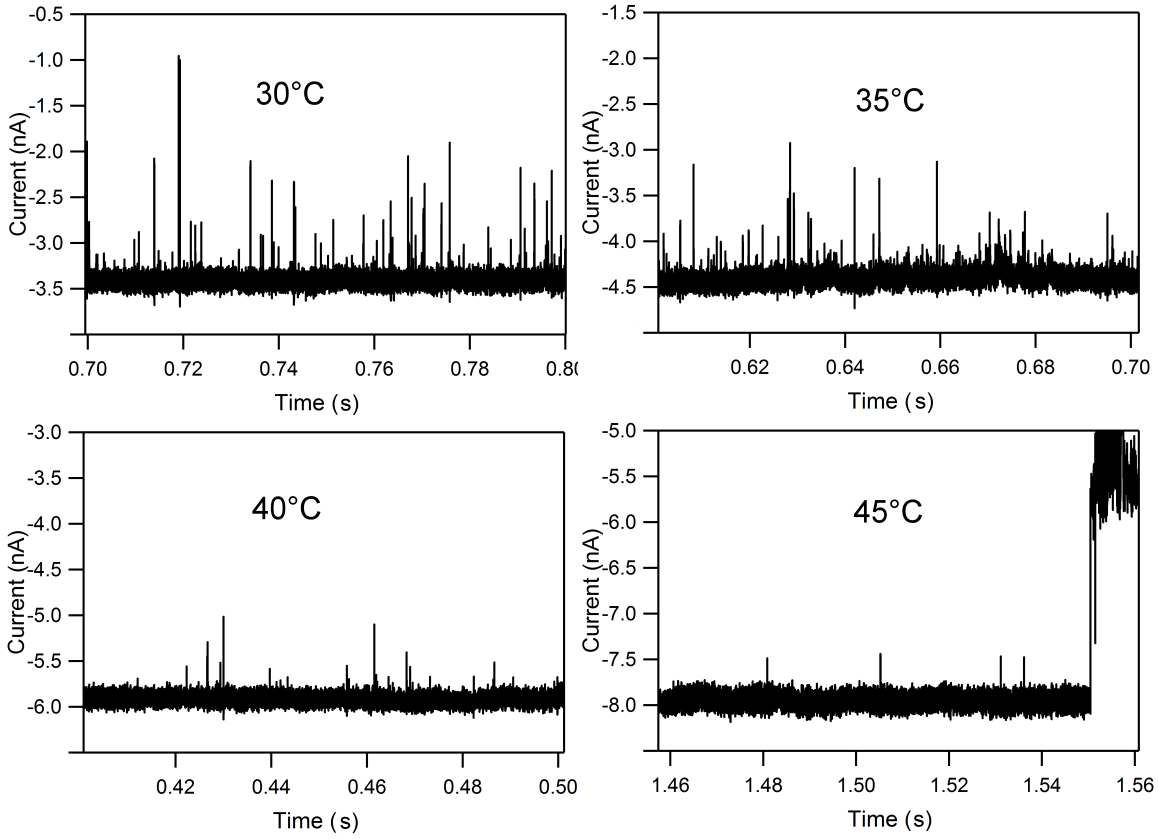

Figure S14: Example trace of GCN4-p1 induced events in a 7 nm diameter nanopore for elevated temperatures. Solution was 1M NaCl, 50 mM potassium phosphate buffer, pH 7.0. Temperature was raised to 48°C and then allowed to cool during which measurements were taken. Traces were taken at 30°C, 35°C, 40°C, and 45°C.